Please add the following claims:

--28. A producer cell producing a retroviral particle, the producer cell comprising a retroviral vector and a DNA construct coding for proteins required for the retroviral vector to be packaged, said retroviral vector comprising a 5' long terminal repeat region of the structure U3-R-U5, one or more sequences selected from coding and non-coding sequences; and a 3' long terminal repeat region, wherein the U3 region comprises a heterologous DNA fragment which is target cell type restricted.

29. A recombinant retroviral particle comprising the retroviral vector according to Claim 1.

30. Recombinant retroviral particle obtained by transfecting a packaging cell line of a retroviral ~~vector [system]~~ kit according to Claim 29 with the retroviral vector according to Claim 29, and culturing ~~the cells~~ under suitable conditions.--

REMARKS

Claim amendments

Claims 2, 3, 4, 6 and 27 have been canceled without prejudice to prosecution of the subject matter claimed in this, related or subsequent applications. Claims 1, 5, 7, 9, 11, 12, 13, 15, 17, 19, 20, 21, 22 and 23 have been amended. Claims 28-30 have been added.

Compliance with 35 U.S.C. 120

In order to comply with 35 U.S.C. § 120, the Examiner has requested that Applicants provide a copy of the Demand and certified copies of the International application and the foreign application to which Applicants claim the benefit of the earlier filing date.

Filed concurrently with this Amendment is a copy of the Demand for PCT/EP95/03445. Applicants are in the process of obtaining certified copies of the foreign application and the International application and will forward a copy to the Examiner upon receipt.

Objection to the disclosure

The Examiner states that the term "advance" on page 17, line 6 of the disclosure should be replaced with the term "advantage". The specification has been amended as suggested by the Examiner.

Rejection of Claims 1-27 under 35 U.S.C. § 112, first paragraph

The Examiner states that the specification is enabling for retroviral vectors which retain the inverted repeat (att) site in the U3 region of the LTR, but does not provide reasonable enablement for retroviral vectors that delete the entire U3 region. Citing the Panganiban *et al.* ('83) reference, the Examiner states that "[i]t is predictable that a retroviral vector that lacks an att site will not integrate into the host genome, and will replicate poorly" (Office Action, page 6).

As amended, the claims relate to a retroviral vector comprising a 3' long terminal repeat (LTR) region comprising a partially deleted U3 region. As the Examiner has indicated, the specification is enabling for retroviral vectors which retain a portion of the U3 region of the LTR, and the portion of the U3 region of a retroviral vector needed for integration into a host genome is well known in the art.

Thus, Applicants have provided an enabling disclosure for the claimed invention, particulary as amended.

Rejection of Claims 1-27 under 35 U.S.C. § 112, second paragraph

Claims 1-27 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

The Examiner states that "Claims 1-27 are indefinite for recitation of the phrase 'capable of', and suggest amending the claims to recite the claimed properties using positive language (Office Action, page 7). The claims have been amended to positively recite a retroviral vector "which undergoes promoter conversion..."

The Examiner states that "Claims 17-24, 26 and 27 are indefinite for recitation of the term 'system' because it is not clear whether the claims are drawn to a method or a kit", and suggests amending the claims to recite "kit" (Office Action, page 7). Claim 27 has been canceled, and Claims 17-24 and 26 have been amended to replace the term "system" with the term "kit".

The Examiner states that there is insufficient antecedent basis for the limitation "said coding sequence for a retroviral protein" in Claim 13. Claim 13 has been amended to include proper antecedent basis.

The Examiner states that "Claims 20, 21 and 27 are indefinite for recitation of the phrase 'in vitro and in vivo' because it is not clear how the method can be performed on both types of cells at the same time and because the phrases 'in vitro' and in vivo' are imprecise terms in the art that may read on cell-free reactions, infection of cultured cells of an animal, and infection of animals" (Office Action, page 7). Applicants have canceled Claim 27, and amended Claim 20 to recite "comprising cells of a human or animal, or isolated cultured cells of a human or an animal", as suggested by the Examiner.

The Examiner states that Claims 7, 11 and 12 are indefinite for reciting "one or more elements of", and suggests deleting the phrase in Claims 11 and 12, and amending Claim 7 to conclude with the phrase "or combinations thereof". Applicants have amended Claims 7, 11 and 12 as suggested by the Examiner.

The Examiner states that Claim 9 is indefinite for reciting "selected from at least one element of the group consisting of a long terminal repeat region", and suggests amending the claim to recite "derived from a retrovirus selected from the group consisting of". Applicants have amended Claim 9 as suggested by the Examiner.

The Examiner states that Claims 12 is indefinite because a comma does not appear after the phrase "alcohol dehydrogenase gene" and that Claim 19 is indefinite because a comma does not appear after the phrase "PA317". Claims 12 and 19 have been amended to insert a comma after the recited phrases.

Rejection of Claims 1, 3, 4, 9, 11, 12, 14, 17, 18, 20, 22, 23, 24, 25 and 27 under 35 U.S.C. § 102(b)

Claims 1, 3, 4, 9, 11, 12, 14, 17, 18, 20, 22, 23, 24, 25 and 27 are rejected under 35 U.S.C. § 102(b) as being anticipated by Faustinella *et al.* in light of Panganiban ('84) and Scarpa *et al.* The Examiner states that Faustinella *et al.* teach the Moloney murine retroviral vector, pS3, which comprises a stop codon in the gag region and a partial deletion of the 3' U3 region into which has been inserted a polylinker with unique cloning sites, and modification of the pS3 vectors wherein the luciferase reporter gene operably linked to a rous sarcoma virus (RSV) promoter or the hygromycin resistance gene operably linked to a herpes simplex thymidine

kinase (TK) promoter has been inserted into the polylinker region. The Examiner also notes that Faustinella *et al.* show that the viral vectors were packaged in the GP+AM12 cell line, and considers it "inherent that the packaging procedure as well as the expression of pS3 in NIH-3T3 cells detailed in Faustinella et al. shows the mRNA and RNA of the retroviral vector were produced during infection of cultured animal cells" (Office Action, page 11). The Examiner cites Scarpa *et al.* as teaching that the mutation of the start codon in the gag region to a stop codon in pS3 results in the absence in translation of the pol gene, and cites Panganiban *et al.* ('84) as teaching that the 3' end of the pol gene encodes the int locus that is required for integration of the reverse transcribed retroviral genome to form a provirus.

As amended, the claims relate to a retroviral vector which undergoes promoter conversion comprising a 5' LTR region of the structure U3-R-U5; one or more sequences selected from coding and non-coding sequences; and a 3' LTR region comprising a partially deleted U3 region which comprises a heterologous DNA fragment which is target cell type restricted. Support for the phrase "target cell type restricted" is, for example, on page 9, lines 25-27 and page 15, lines 8-17 and lines 19-34 of the specification. None of the references cited in support of the 102(b) rejection teach a retroviral vector comprising a heterologous DNA fragment which is target cell type restricted.

Faustinella *et al.* teach a retroviral vector wherein the U3 region of the 3' LTR comprises the luciferase gene driven by the RSV promoter or the hygromycin resistance gene driven by the herpes simplex TK promoter. The genes and promoters (i.e., heterologous DNA fragments) used in the retroviral vectors of Faustinella *et al.* are not target cell type restricted. Applicants are filing the abstract of Gorman *et al, Proc. Natl. Acad. Sci., USA, 79(22)*:6777-6781 (1979) as Exhibit A, which provides evidence that the RSV promoter is unselective in that it is active in many cell types (e.g., monkey kidney cells, chicken embryo fibroblast cells, Chinese hamster ovary cells). In addition, Applicants are filing two pages from the CLONTECH catalogue as Exhibit B, which provides evidence that the herpes simplex TK promoter regularly drives expression of the hygromycin resistant gene mediating selection of "a broad range of human host cells" (see page 1 of Exhibit B). Faustinella *et al.* do not teach or suggest the introduction of a DNA fragment with cell type restricted properties within the U3 region of a retroviral vector.

Scarpa *et al.* characterized recombinant helper retroviruses from Moloney-based vectors in ecotropic and amphotropic packaging cell lines. Panganiban *et al.* ('84) performed experiments which indicate that the 3' end of the *pol* gene of the spleen necrosis virus encodes a

polypeptide required for DNA integration through interaction with the *att* site. There is no discussion in either the Scarpa *et al.* or Panganiban *et al.* reference regarding a retroviral vector comprising a heterologous DNA fragment which is target cell type restricted.

Thus, the teachings of Faustinella *et al.* in light of the teachings in the Panganiban *et al.* and Scarpa *et al.* references do not anticipate Applicants' claimed invention, particularly as amended.

Rejection of Claims 1, 3-9, 16, 17 and 19 under 35 U.S.C. § 103(a)

Claims 1, 3-9, 16, 17 and 19 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Faustinella *et al.* in view of Couture *et al.* in view of Mee *et al.* The Examiner summarizes Faustinella *et al.* as described for the 102(b) rejection, but notes that Faustinella *et al.* do not show heterologous DNA consisting of regulatory elements and promoters recited in Claims 5-8, the LTR regions recited in Claim 9 other than the murine leukemia LTR, the regulatable elements that are explicitly regulated by trans acting molecules, the packaging cells of Claim 19, the BAG vector, or a pharmaceutical comprising a retroviral vector. The Examiner states that Couture *et al.* teach a retroviral vector comprising a substitution of a portion of the 3' U3 region with the corresponding region of 5 murine retroviruses; that after packaging, the substituted U3 region appears at the 5' LTR and serves as a promoter for vector genes; that different LTR constructs were preferentially expressed in specific cell types; that promoter suppression or interference may occur within retroviral vectors containing internal promoter elements; that retroviral vectors with target cell specificity have utility in gene therapy protocols; and the use of packaging cells lines PA 317 and GP&E86. The Examiner states that Mee *et al.* teach a retroviral vector comprising a mouse mammary tumor virus LTR and that the LTR expressed a gene after induction with dexamethasone. The Examiner states that Mee *et al.* teach that their vector is a potentially tool for the manipulation of gene expression in a variety of cell types. It is the Examiner's opinion that:

> [i]t would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the vector of Faustinella *et al.* by the use of the LTR regions of Mee et al. because Couture et al. show that insertion of a promoter region in a deleted 3' U3 region of a retroviral vector results in expression of vector genes under the control of the inserted promoter in a cell type specific manner, and that internal promoters may not function properly in a retroviral vector, and that target cell specific retroviral vectors have utility in gene therapy protocols, and because Mee et al. show that their LTR promoter may be used to manipulate gene expression in a variety of cell types. It would have been further

> obvious to use packaging cell lines PA317 and GP&E86 because Couture et al. shows that they may be used to package retroviral vectors (Office Action, pages 15-16).

Applicants respectfully disagree. The claims, as amended, relate to a retroviral vector which undergoes promoter conversion comprising a 5' LTR region of the structure U3-R-U5; one or more sequences selected from coding and non-coding sequences; and a 3' LTR region comprising a partially deleted U3 region which comprises a heterologous DNA fragment which is target cell type restricted. Applicants clearly teach and demonstrate in the specification as filed that promoters which direct tissue specific expression (e.g., the WAP promoter) of the heterologous DNA can be inserted into the claimed retroviral vectors, thereby limiting expression of therapeutic genes to specific target cells (e.g., specification, page 15, lines 8-17; Example 1, pages 22-24).

As discussed above, Faustinella *et al.* do not teach a retroviral vector comprising a heterologous DNA fragment which is target cell type restricted. The remaining references do not provide the teaching that is lacking in the Faustinella *et al.* reference to render Applicants' claimed invention obvious.

Couture *et al.* replaced the U3 promoter of the Moloney murine leukemia virus (MoLV) with the U3 region from five related murine retroviruses and assessed the promoter/enhancer strength of the chimeric LTRs (chLTR) in both mouse and human cell lines and primary cells. Couture *et al.* found

> clear differences in expression <u>levels</u> occur for various retroviral LTRs when their respective U3 enhancer/promoter regions are placed in the context of a common MoMLV LTR and a common retroviral vector (Couture *et al.*, page 674, column 2, emphasis added).

Couture *et al.* teach that in the murine cell lines tested, the Harvey murine sarcoma virus chLTR vector was "slightly more active than the MoLV chLTR vector", in two human cell lines tested, the myeloproliferative sarcoma virus chLTR was most active and in two other human cell lines tested, the SL3-3 chLTR was most active. The promoter/enhancer elements described in the Couture *et al.* reference, however, directed expression of the reporter gene in <u>all</u> the human and mouse cell lines tested. There is no discussion in the Couture *et al.* reference regarding the introduction of a DNA fragment with cell type restricted properties within the U3 region of a retroviral vector. That is, Couture *et al.* do not teach or suggest introducing a DNA fragment which includes a promoter that directs target cell type restricted gene expression into the U3

region of a retroviral vector. The teaching in the Couture *et al.* reference is limited to developing vectors "with the greatest expression level in a particular cell target" (Couture *et al.*, page 674, column 2, emphasis added). In contrast, Applicants claimed invention is directed to a retroviral vector which undergoes promoter conversion comprising a 5' LTR region of the structure U3-R-U5; one or more sequences selected from coding and non-coding sequences; and a 3' LTR region comprising a partially deleted U3 region which comprises a heterologous DNA fragment which is target cell type restricted.

Mee *et al.* teach the construction and properties of a self-inactivating (SIN) retroviral vector containing a hormonally regulated transcriptional element. In particular, Mee *et al* disabled the 3' LTR of a retroviral vector and cloned the HRE inducible promoter of the MMTV and the *aph* gene directly between the LTRs of the provirus (Mee *et al.*, pages 289-290). In contrast, Applicants teach a retroviral vector having a partially deleted U3 region wherein the vector does not undergo self-inactivation. There is clearly no discussion in the Mee *et al.* reference regarding the introduction of a DNA fragment with cell type restricted properties within the U3 region of a retroviral vector.

Where the claimed invention is rejected as obvious in view of a combination of references, § 103 requires both (1) that "the prior art would have suggested to the person of ordinary skill in the art that they should . . . carry out the claimed process"; and (2) that the prior art should establish a reasonable expectation of success (*In re Vaeck*, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991)). "Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure." *Id.* There is no particular teaching in the art cited directing the skilled person to construct a retroviral vector which undergoes promoter conversion comprising a 5' LTR region of the structure U3-R-U5; one or more sequences selected from coding and non-coding sequences; and a 3' LTR region comprising a partially deleted U3 region which comprises a heterologous DNA fragment which is target cell type restricted.

Faustinella *et al.*, Couture *et al.* and Mee *et al.* either alone or in combination do not teach or suggest carrying out Applicants' claimed invention. That is, the combined teachings of the cited references do not teach or suggest introducing a DNA fragment into the U3 region of the 3' LTR of a retroviral for cell type restricted targeting of therapeutic genes. At most, the combined teachings of Faustinella *et al.* and Couture *et al.* would lead the skilled practitioner to introduce target cell type unspecific promoters, as disclosed in Couture *et al.*, into multiple cloning sites in the 3' LTR

of a retroviral vector, as disclosed in Faustinella *et al.* Mee *et al.* disclose a self-inactivating retroviral vector wherein the U3 region of the 3' LTR of a retroviral vector has been deleted to functionally inactivate the retroviral promoter and thus prevent further replication in the target cell. Since the retroviral vector construct according to Faustinella *et al.* necessarily requires the U3 region of the 3' LTR, the skilled person would not be motivated to combine the teaching of Mee *et al.* with the teaching of Faustinella *et al.* The combined teachings of Couture *et al.* and Mee *et al.* would lead the person skilled in the art to introduce the target cell type nonspecific promoters disclosed in Couture *et al* into a SIN vector for purposes of safe gene transfer as disclosed in Mee *et al.*

Thus, the teachings of the cited references, either alone or in combination, do not render obvious Applicants' claimed invention, particularly as amended.

Rejection of Claims 1, 10, 11 and 12 under 35 U.S.C. § 103(a)

Claims 1, 10, 11 and 12 are rejected under 35 USC § 103 (a) as being unpatentable over Faustinella *et al.* in view of Price *et al.* The Examiner applies Faustinella *et al.* as discussed above, and states that Price *et al.* "show a BAG retroviral vector comprising a beta galactosidase reporter gene, and that the vector can be used to identify cells and progeny of cells infected with the vector" (Office Action, page 16). It is the Examiner's opinion that

> [i] would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the vector of Faustinella et al. by basing the construction on a BAG vector of Price et al. because Price et al. shows that a vector with a beta-galactosidase reporter gene may be used to identify cells and progeny of cells infected with the vector (Office Action, page 16).

Applicants respectfully disagree. As discussed above, where the claimed invention is rejected as obvious in view of a combination of references, § 103 requires "[b]oth the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure." (*In re Vaeck*, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991)). Faustinella *et al.* do not teach a retroviral vector comprising a heterologous DNA fragment which is target cell type restricted. The teaching in the Price *et al.* reference does not provide the teaching that is lacking in the Faustinella *et al.* reference to render Applicants' claimed invention obvious. Price *et al.* applied a β-gal-transducing vector, BAG, "to the study of neural lineage *in vivo* and in culture and have been able to mark cells in both cases" (Price *et al.*, page 158, column 2). There is clearly no discussion in the Price *et al.* reference regarding the introduction of a DNA fragment with cell type restricted

properties within the U3 region of a retroviral vector. Therefore, at most, the combined teachings of the Faustinella *et al.* and Price *et al.* references would lead a person of skill in the art to introduce multiple cloning sites in the 3' LTR of the BAG retroviral vector. The combined teachings of Faustinella *et al.* and Price *et al.*, however, do not even suggest introducing a DNA fragment into the U3 region of the 3' LTR of a retroviral for cell type restricted targeting of therapeutic genes.

Thus, the teachings of the cited references, either alone or in combination, do not render obvious Applicants' claimed invention, particularly as amended.

Rejection of Claims 1, 3, 4, 15, 17, 20, 21, 22, 26 and 27 under 35 U. S.C. § 103 (a)

Claims 1, 3, 4, 15, 17, 20, 21, 22, 26 and 27 rejected under 35 USC § 103(a) as being unpatentable over Faustinella *et al.* in view of Longmore *et al.* and Kay *et al.* The Examiner applies Faustinella *et al.* as discussed above. The Examiner cites Longmore *et al.* as showing that mice infected with a retroviral vector expressing the erythropoietin receptor had increased platelet counts and splenic megakaryocytes, and cites Kay *et al.* as teaching that hemophiliac dogs infected with a retroviral vector expressing factor IX improved levels of clotting and thromboplastin for greater than 5 months after treatment" (Office Action, page 17). It is the Examiner's opinion that

> [i]t would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the vector of Faustinella *et al.* to express a therapeutic protein because both Kay et al. and Longmore et al. show that retroviral vectors may be used to express therapeutically effective levels of a recombinant protein in an animal (Office Action, page 17).

Applicants respectfully disagree. As discussed above, Faustinella *et al.* do not teach a retroviral vector comprising a heterologous DNA fragment which is target cell type restricted. The teachings in the Longmore *et al.* and Kay *et al.* references do not provide the teaching that is lacking in the Faustinella *et al.* reference to render Applicants' claimed invention obvious.

Longmore *et al.* infected mice with a recombinant spleen focus-forming retrovirus (SFFV) expressing an oncogenic erythropoietin (Epo) receptor (EpoR) and showed that a relationship between erythropoiesis and thrombopoiesis can exist at the level of the Epo-EpoR signalling pathway. In addition, Longmore *et al.* teach that the SFV-based vectors "may be excellent vehicles for the introduction of genes into multipotent, hemapoietic progenitors, in vitro" (Longmore *et al.*, abstract).

Using a hemophilia B dog model, Kay *et al.* determined that a method for hepatic gene transfer *in vivo* by the direct infusion of recombinant retroviral vectors into the portal vasculature which results in the persistent expression of exogenous genes may be feasible for the treatment of hemophilia B patients. Kay *et al.* used an amphotropic retroviral vector that encoded the canine factor IX complementary DNA.

There is no discussion in either the Longmore *et al.* or Kay *et al.* reference regarding a retroviral vector comprising a heterologous DNA fragment which is target cell type restricted. Thus, at most, the combined teachings of Faustinella *et al.*, Longmore *et al.* and Kay *et al.* would lead a person of skill in the art to introduce the EpoR DNA, as disclosed in the Longmore *et al.* reference, or factor IX DNA, as disclosed in the Kay *et al.* reference, into multiple cloning sites in the 3' LTR of a retroviral vector, as disclosed in the Faustinella *et al.* reference. The combined teachings of Faustinella *et al.*, Longmore *et al.* and Kay *et al.*, however, do not even suggest introducing a DNA fragment into the U3 region of the 3' LTR of a retroviral for cell type restricted targeting of therapeutic genes.

Thus, the teachings of the cited references, either alone or in combination, do not render obvious Applicants' claimed invention, particularly as amended.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (781) 861-6240.

Respectfully submitted,

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